(Boehringer) according to a commonly used method (Molecular Cloning. A laboratory manual/2nd edition (1989), p7.46-7.50, Cold Spring Harbor Laboratory Press). Then, the nylon membrane was subjected to ultraviolet light irradiation to fix the RNA on the filter.

An oligodeoxynucleotide (gtcacagaattttgagaccca; SEQ ID NO: 2) specific to EF1α was obtained from GIBCO. Labeling of the oligonucleotide by terminal transferase was performed in the same manner as described above. Hybridization treatment of the EF1α RNA-immobilized filter with the labeled oligodeoxynucleotide and detection of hybridization by chemical luminescence method was performed in the same manner as described above. The results are shown in Figure 2. A single band was detected, which corresponds to the expected size (1.0 kb). As clearly seen in Figure 2, the hybridization probe according to the present invention has sufficient sensitivity and specificity to detect the target mRNA on the blot. Thus, it is confirmed that detection for gene expression profiling and SNP can be achieved based on this principle.

Industrial Applicability

Hybridization due to a nucleotide sequence newly added for labeling can be effectively prevented according to the present invention. Further, the nucleotides used in the present invention can be substrates for terminal transferase, and therefore label can be readily introduced into a nucleic acid sequence by adding the nucleotides as substrates in the 3'-tailing labeling method.

The nucleotides (or nucleotide derivatives) exhibiting weak affinity in base pairing, which are used in the present invention, can function as nucleotides for labeling or as spacer nucleotides, and thus it is possible to achieve the efficient introduction of labeled nucleotides and to provide labeled oligonucleotide with high specific activity. Thus, according to the present invention, both the two objectives are achievable; namely inhibition of nonspecific hybridization reaction and preparation of labeled oligonucleotide with high specific activity. In other words, the S/N ratio in hybridization can be readily improved according to the present invention.

CLAIMS

1. A hybridization probe in which a nucleotide sequence comprising labeled nucleotides or nucleotide derivatives is added to a DNA to be labeled, the added nucleotide sequence

a) comprising nucleotides and/or nucleotide derivatives having weaker affinity of hydrogen bonding in base pairing with bases of the target nucleotide sequence when compared with those of hydrogen bonding in an a/t pair, in an a/u pair, and in a g/c pair; and

b) being introduced into the DNA to be labeled through nucleotide-adding reaction with terminal transferase.

2. The hybridization probe of claim 1, wherein the nucleotides of a) are inosinic acids.

3. The hybridization probe of claim 2, wherein the added nucleotide sequence comprises labeled nucleotides or nucleotide derivatives and unlabeled inosinic acids or derivatives thereof.

4. The hybridization probe of claim 3, wherein the labeled nucleotides or nucleotide derivatives are labeled inosinic acids or inosinic acid derivatives.

5. The hybridization probe of claim 1, wherein the added nucleotide sequence *per se* is incapable of hybridizing to any nucleotide sequences under stringent hybridization conditions for the DNA to be labeled.

6. A method for detecting, with the hybridization probe of any one of claims 1 to 5, a nucleic acid having a nucleotide sequence complementary to the DNA to be labeled.

7. The method of claim 6, wherein RNA or cDNA library is to be detected.

8. A method for labeling a DNA by 3'-tailing with terminal transferase, wherein nucleotides and/or nucleotide derivatives having weaker affinity of hydrogen bonding in base pairing when compared with those of hydrogen bonding in an a/t pair, in an a/u pair, and in a g/c pair and which can be substrates in nucleotide-adding reaction with terminal transferase are used as substrates.

9. The method of claim 8, wherein the nucleotide is

deoxyinosine 5'-triphosphate.

10. The method of claim 8, wherein the nucleotides and/or nucleotide derivatives having weaker affinity in base pairing are mixed with labeled nucleotides or nucleotide derivatives and used as the substrates.

11. A kit for synthesizing a hybridization probe, the kit comprising

i) nucleotides and/or nucleotide derivatives

(a) having weaker affinity of hydrogen bonding in base pairing with bases of the target nucleotide sequence when compared with those of hydrogen bonding in an a/t pair, in an a/u pair, and in a g/c pair; and

(b) being introduced into a DNA to be labeled through nucleotide-adding reaction with terminal transferase;

ii) labeled nucleotides or nucleotide derivatives; and

iii) terminal transferase.

12. A method for preventing hybridization of a hybridization probe in which a nucleotide sequence comprising labeled nucleotides is added to a DNA to be labeled, the hybridization non-specific to the sequence of the DNA to be labeled, wherein the nucleotides and/or nucleotide derivatives having weaker affinity of hydrogen bonding in base pairing when compared with those of hydrogen bonding in an a/t pair, in an a/u pair, and in a g/c pair are inserted into the added nucleotide sequence.